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Composition of *Myroxylon pereirae* Resin and Colophonium for Patch Testing

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ABSTRACT

Background: In patch testing, co-reactivity between *Myroxylon pereirae* resin, colophonium and propolis is well recognised. One of the possible explanations is that these materials have common allergenic ingredients.

Objectives: To identify the main ingredients in *M. pereirae* resin and colophonium samples used in the preparation of commercial patch test allergens and to compare their compositions with each other as well as with propolis.

Materials and Methods: Analyses were performed on *M. pereirae* resin and colophonium samples using gas chromatography–mass spectrometry/flame ionisation detection of the volatile components obtained by headspace SPME (solid phase microextraction).

Results: The main ingredients in *M. pereirae* resin were benzyl benzoate, (*E*)-nerolidol, benzoic acid, benzyl alcohol, (*E*)-cinnamic acid, vanillin and (*E*)-benzyl cinnamate. In colophonium, longifolene, caryophyllene oxide, acetone + formic acid, α-terpineol and δ-cadinene + calamenene were the major constituents.

Conclusions: The major ingredients of the volatile fractions of *M. pereirae* resin and colophonium are quite different; common haptens in volatile ingredients cannot readily explain co-reactivity. *M. pereirae* resin has cinnamic acid- and benzoic acid derivatives in common with propolis and in addition (*E*)-nerolidol and vanillin with Brazilian propolis and benzyl alcohol with Chinese propolis. Colophonium shares various ingredients with Brazilian propolis but few with the Chinese variety.

1 | Introduction

Co-reactivity between propolis, *Myroxylon pereirae* resin (MPR) (balsam of Peru), fragrances, colophonium and essential oils is well recognised [1–5]. One of the possible explanations is that these materials have common allergenic ingredients. In our previous study, we examined the major components of the volatile fraction of propolis from Brazil and propolis from China used for preparing commercial patch test preparations [6]. Here we report the results of analytical investigations of such samples of MPR and colophonium.

2 | Materials and Methods

2.1 | Materials

The materials analysed were samples of MPR (viscous liquids) and colophonium (powder) used by SmartPractice (www.smartpracticeeurope.com) and Chemotechnique (www.chemotechnique.se) to prepare their test allergens; the samples were kindly donated by these companies. SmartPractice commercialises two test materials of the brand Allergeaze: Balsam of Peru (MPR) 25% pet. (item NA12) and colophony 20% pet.

(item NA24). Chemotechnique commercialises Peru balsam 25% pet. (article no. B-001) and colophonium 20% pet. (article no. C.020).

2.2 | Methods

A general description of analyses with gas chromatography—mass spectrometry/flame ionisation detection (GC–MS/FID) of volatile components obtained by headspace SPME and the technical details of the experiments performed are provided in Supporting Information Data S7.

3 | Results

3.1 | Composition of *M. pereirae* Samples

3.1.1 | MPR Chemotechnique

The chromatogram of MPR Chemotechnique is shown in Supporting Information Data S3. The number of detected peaks was 54, of which 25 were identified, accounting for 98.95% of the total peak area. The 15 main components are shown in the left 2 columns of Table 1. Together they comprise 98.27% of the total peak area. The Top-7 have a share of 96.33% peak area.

TABLE 1 | Main components of two samples of *Myroxylon pereirae* resin.

Ingredients chemotechnique	PA (%)	Ingredients allergeaze	PA (%)
Benzyl benzoate	62.59	Benzyl benzoate	64.50
(*E*)-Nerolidol	14.72	(*E*)-Nerolidol	15.54
Benzoic acid	8.87	Benzoic acid	7.79
Benzyl alcohol	3.42	Benzyl alcohol	2.60
(*E*)-Cinnamic acid	2.62	(*E*)-Benzyl cinnamate	2.49
Vanillin	2.06	Vanillin	2.01
(*E*)-Benzyl cinnamate	2.05	(*E*)-Cinnamic acid	1.69
Benzaldehyde	0.58	Benzaldehyde	0.46
α-Pinene	0.27	Ethyl benzoate	0.35
(*Z*)-Benzyl cinnamate	0.22	(*Z*)-Benzyl cinnamate	0.28
Styrene	0.22	Styrene	0.18
(*E*,*E*)-α-Farnesene	0.18	(*Z*,*E*)-α-Farnesene	0.17
(*E*)-β-Farnesene	0.17	α-Pinene	0.14
Ethyl benzoate	0.16	(*E*)-β-Farnesene	0.10
(*Z*,*E*)-α-Farnesene	0.14	(*E*,*E*)-α-Farnesene	0.09
Sum of 15 main ingredients	98.27%	Sum of 15 main ingredients	98.39%

Abbreviation: PA, peak area.

The data of all 25 chemicals identified in MPR Chemotechnique with retention times and retention indices, percentages of peak areas and CAS numbers is shown in the Supporting Information Data S2, Table 1).

3.1.2 | MPR Allergeaze

The chromatogram of MPR Allergeaze is shown in Supporting Information Data S4, Figure 2. The number of detected peaks was 61, of which 24 were identified, accounting for 98.84% of the total peak area. The 15 main components are shown in the right 2 columns of Table 1. Together they comprise 98.39% of the total peak area. The Top-7 have a share of 96.62% peak area. The data of all 24 chemicals identified in MPR Allergeaze with retention times and retention indices, percentages of peak areas and CAS numbers is shown in Supporting Information Data S2, Table 2.

3.1.3 | Comparison Between the 2 MPR Samples

As shown in Table 1, the MPR samples from Chemotechnique and Allergeaze exhibit a remarkable similarity in composition. Both samples contain the same 15 major components and the concentrations, based on peak areas, are closely aligned, both for individual substances and for the combined total of all 15.

3.2 | Composition of Colophonium Samples

3.2.1 | Colophonium Chemotechnique

The chromatogram of Colophonium Chemotechnique is shown in the Supporting Information Data S5, Figure 3. The number of detected peaks was 260, of which 69 were identified, accounting for 72.08% of the total peak area. The 15 main components are shown in the left 2 columns of Table 2. Together they comprise 57.07% of the total peak area. The data of all 69 (combinations of) chemicals identified in Colophonium Chemotechnique with retention times and retention indices, percentages of peak areas and CAS numbers is shown in Supporting Information Data S2, Table 3.

Colophonium Chemotechnique contained far less volatile material than the colophonium from Allergeaze. During the enrichment at 80°C, the powder sample melted to a viscous liquid that recrystallised at room temperature (the material from Allergeaze remained a powder).

3.2.2 | Colophonium Allergeaze

The chromatogram of MPR Allergeaze is shown in Supporting Information Data S6, Figure 4. The number of detected peaks was 252, of which 63 were identified, accounting for 70.99% of the total peak area. The 15 main components are shown in the right 2 columns of Table 2. Together they comprise 56.67% of the total peak area. The data of all 63 (combinations of) chemicals identified in Colophony Allergeaze with retention times and retention indices, percentages of peak areas and CAS numbers is shown in Supporting Information Data S2, Table 4).

TABLE 2 | Main components of two samples of Colophonium.

Ingredients chemotechnique	PA (%)	Ingredients allergeaze	PA (%)
Longifolene	10.07	Longifolene	17.83
Caryophyllene oxide	9.22	Caryophyllene oxide	8.46
Acetone + formic acid	8.11	δ-Cadinene + calamenene	6.80
α-Terpineol	6.52	α-Terpineol	5.53
δ-Cadinene + calamenene	5.47	Acetone + formic acid	3.48
Nonanoic acid	3.60	Acetic acid	3.45
Acetic acid	3.06	Humulene epoxide II	1.60
Octanoic acid	2.08	α-Copaene	1.47
(*E*)-β-Caryophyllene	1.98	Longicamphenylone + unknown	1.26
Humulene epoxide II	1.57	Benzyl benzoate	1.24
Limonene	1.52	α-Calacorene	1.20
α-Copaene	1.24	Longiborneol	1.15
α-Muurolene	1.05	α-Muurolene	1.11
Longicamphenylone + unknown	0.80	Curcumene + γ-muurolene	1.05
1-epi-Cubenol	0.78	1-epi-Cubenol	1.04
Sum of 15 main ingredients	57.07%	Sum of 15 main ingredients	56.67%

Abbreviation: PA, peak area.

3.2.3 | Comparison Between the 2 Colophonium Resin Samples

Colophonium is a complex substance in which there may be over 250 volatile chemicals present. There are many similarities in the samples from Chemotechnique and Allergeaze. The five chemicals with the largest peak areas are identical, albeit in a different order. The total peak area of these five in both samples is around 40%. Of the other 10 of the Top-15 chemicals, 6 (acetic acid, humulene epoxide II, α-copaene, α-muurolene, longicamphenylone and 1-epi-cubenol) are present in both samples with generally corresponding peak area percentages.

3.3 | Comparison Between the Compositions of MPR and Colophonium

Comparison between the compositions of MPR (Table 1) and colophonium (Table 2) shows major differences. Of the 15 major ingredients in MPR, only one (benzyl benzoate) is also present in the Top-15 of colophonium, in a low concentration of 1.24% (peak area).

3.4 | Comparison Between the Compositions of Propolis and Those of MPR and Colophonium

In our previous study, we have analysed the compositions of the volatile fractions of Brazilian propolis (propolis B, Allergeaze) and Chinese propolis from Allergeaze and Chemotechnique [6]. An alphabetical list of all chemicals identified in the seven samples (three propolis, two MPR, two colophonium) with their percentages peak area is shown in Supporting Information Data S1.

The (chemically related) ingredients from the Top-15 that propolis and MPR have in common are shown in Table 3. For Brazilian propolis, the most important components shared are cinnamic acid-derivatives, (*E*)-nerolidol, benzoic acid (derivatives) and vanillin. For Chinese propolis and MPR, common or chemically related ingredients are cinnamic acid-derivatives, benzoic acid (−derivatives) and benzyl alcohol.

The (chemically related) ingredients from the Top-15 that propolis and colophonium have in common are shown in Table 4. For Brazilian propolis, the most important shared components are benzoic acid (−derivatives), δ-cadinene + calamenene, acetic acid, α-curcumene + γ-muurolene, caryophyllene (oxide), α-copaene and α-muurolene. Chinese propolis and colophonium only have two components in the Top-15 in common: curcumene and acetic acid.

4 | Discussion

Co-reactivity between Chinese propolis, MPR, fragrances, colophonium and essential oils is well known [1–5]. Brazilian propolis, which has been available for patch testing since 2019 (Allergeaze, propolis B), also shows co-reactivities to fragrances and indicators of fragrance sensitivity [7–10]. One of the possible explanations is that these substances share common (allergenic) ingredients. Indeed, Chinese propolis and MPR can have at least 26 ingredients in common, among which chemicals that are known to have caused sensitization and allergic contact

TABLE 3 | Common and chemically related ingredients in propolis and *Myroxylon pereirae* resin.

Propolis	% PA	*Myroxylon pereirae* resin[a]	% PA
Brazilian propolis (Allergeaze)			
Cinnamic acid-derivatives		Cinnamic acid-derivatives	
Hydrocinnamic acid	16.9	(*E*)-Cinnamic acid	1.69–2.62
		(*E*)-Benzyl cinnamate	2.05–2.49
		(Z)-Benzyl cinnamate	0.22–0.28
(*E*)-Nerolidol	7.41	(*E*)-Nerolidol	14.72–15.54
Benzoic acid-derivatives		Benzoic acid-derivatives	
Benzoic acid + benzyl acetate +	3.22	Benzoic acid	7.79–8.87
4-ethylphenol	64.50	Benzyl benzoate	62.59–64.50
β-Bourbonene + **vanillin**	1.85	Vanillin	2.01–2.06
Chinese propolis (Allergeaze and Chemotechnique combined)			
Cinnamic acid-derivatives		Cinnamic acid-derivatives	
(*E*)-Cinnamyl alcohol	8.08–24.96	(*E*)-Benzyl cinnamate	2.05–2.49
(*E*)-Cinnamyl acetate	3.48	(*E*)-Cinnamic acid	1.69–2.62
(*E*)-Cinnamaldehyde	1.68–2.14	(*Z*)-Benzyl cinnamate	0.22–0.28
Benzoic acid-derivatives		Benzoic acid-derivatives	
Benzoic acid + benzyl acetate	4.70	Benzyl benzoate	62.59–64.50
		Benzoic acid	7.79–8.87
		Ethyl benzoate	0.16–0.35
Benzyl alcohol	2.04	Benzyl alcohol	2.60–3.42

Abbreviation: PA, peak area.
[a]From Chemotechnique and from Allergeaze combined. Chemicals in mixtures in propolis that are also present in Myroxylon pereirae resin in bold.

TABLE 4 | Common and chemically related ingredients in propolis and colophony.

Propolis	% PA	Colophonium[a]	% PA
Brazilian propolis			
Benzoic acid-derivatives		Benzoic acid-derivatives	
Benzoic acid + benzyl acetate + 4-ethylphenol	3.22	Benzyl benzoate	1.24
δ-Cadinene + calamenene	3.11	δ-Cadinene + calamenene	5.47–6.80
Acetic acid	2.90	Acetic acid	3.06–3.45
α-Curcumene + γ-muurolene	2.46	Curcumene + γ-muurolene	1.05
α-Muurolene + α-selinene	1.44	α-Muurolene	1.05–1.11
Caryophyllene oxide	2.38	Caryophyllene oxide	8.46–9.22
β-Caryophyllene	1.46	β-Caryophyllene	1.98
α-Copaene	1.62	α-Copaene	1.24–1.47
Chinese propolis (Allergeaze and Chemotechnique combined)			
α-Curcumene	8.77–8.81	**Curcumene** + γ-muurolene	1.05
Acetic acid	2.26	Acetic acid	3.45–3.60

Abbreviation: PA, peak area.
[a]From Chemotechnique and from Allergeaze combined. Chemicals in mixtures in propolis that are also present in Colophonium and vice versa in bold.

dermatitis, including benzoic acid and -derivatives (benzyl benzoate, coniferyl benzoate), cinnamic acid and -derivatives (benzyl cinnamate, cinnamyl alcohol), benzyl salicylate, benzyl alcohol, eugenol, nerolidol and vanillin [1–4]. Propolis, MPR and colophonium are plant-derived substances, the composition of which may be very variable, depending inter alia on their source materials and geographical origin. Therefore, to be able to accurately evaluate co-reactivities observed in patch test studies, knowledge about the composition of the substances used in commercial propolis, MPR and colophonium patch test materials is essential. The manufacturers of these products, Chemotechnique (brand Chemotechnique) and SmartPractice (brand Allergeaze) could not provide detailed information.

In a previous investigation, we analysed the compositions of the volatile fractions of Brazilian and Chinese propolis from both manufacturers [6]. It was found that the composition of the Brazilian propolis was very different from the Chinese samples. In the current study, the compositions of MPR and colophonium from Chemotechnique and Allergeaze were investigated. The major components identified in MPR were benzyl benzoate, (*E*)-nerolidol, benzoic acid, benzyl alcohol, (*E*)-cinnamic acid, vanillin and (*E*)-benzyl cinnamate, which in both samples accounted for > 96% of the peak area in the chromatograms. The compositions of the two Chinese samples showed great similarities. In colophonium, longifolene, caryophyllene oxide, acetone + formic acid, α-terpineol and δ-cadinene + calamenene were the major ingredients in the volatile fraction. The compositions of the Chemotechnique and Allergeaze samples also showed many similarities.

When comparing the compositions of Chinese propolis, Brazilian propolis, MPR and colophonium to identify common ingredients or chemically related components (which may of might cross-react), Brazilian propolis and MPR share some cinnamic acid derivatives, (*E*)-nerolidol, benzoic acid (−derivatives) and vanillin (Table 3). For Chinese propolis and MPR, common or chemically related ingredients were also cinnamic and benzoic acid (−derivatives) and benzyl alcohol (Table 3).

With colophonium, Brazilian propolis shares benzoic acid (−derivatives), δ-cadinene + calamenene, acetic acid, α-curcumene + γ-muurolene, caryophyllene (oxide), α-copaene and α-muurolene (Table 4). Chinese propolis and colophonium only have two components in the Top-15 in common, curcumene and acetic acid (Table 4).

It is unknown whether these chemicals, present in two or three of the substances, are responsible for the observed co-reactivities. In Chinese propolis, caffeic acid and its esters are considered to be the main allergenic ingredients; cinnamic acid and its esters (cinnamyl, benzyl, methyl) have been identified as (possible) culprits also, but less often [1, 4]. In patients allergic to MPR, positive patch tests have been observed in > 10% of the allergic individuals to its ingredients coniferyl benzoate, isoeugenol, eugenol, cinnamyl alcohol, cinnamic acid, cinnamyl cinnamate, cinnamal, benzoic acid, benzyl alcohol, benzyl cinnamate, vanillin, coniferyl alcohol and benzyl benzoate. The major chemicals in colophony identified as contact allergens are the oxidation products of abietic and dehydroabietic acid [4, 5]. Finally, the sensitizers in Brazilian propolis are (completely) unknown, as allergy to this propolis variety has only been detected very recently [7–10]. The relevant literature on the (possible) allergens in Chinese propolis has been reviewed in Refs [1, 4]; that of MPR is fully reviewed in Refs. [2, 3] and the literature on the allergens in colophonium has been summarised in Refs. [4, 5].

Ingredient patch testing in patients reacting to propolis, MPR, colophonium, or a combination may result in more information on the allergenic ingredients and the cause of co-reactivity. The data presented here may give some direction in composing such ingredient patch test series, keeping in mind that the information from this study on the ingredients of propolis, MPR and colophonium is limited to their volatile fractions.

4.1 | Conclusions

The major ingredients of the volatile fractions of MPR and colophonium are quite different; common haptens in volatile ingredients cannot readily explain co-reactivity. MPR has cinnamic acid- and benzoic acid-derivatives in common with both propolis varieties and in addition (*E*)-nerolidol and vanillin with Brazilian propolis and benzyl alcohol with Chinese propolis. Colophonium shares various ingredients with Brazilian propolis but few with Chinese propolis.

4.2 | Recommendations for Further Research

For identifying the (possible common) sensitizers in MPR, colophonium and propolis, we suggest that allergic patients be tested in a second session with the main ingredients found in this study (Table 1 [MPR] and Table 2 [colophonium]) and in our previous investigation [6] ([propolis]).

The compositions of the nonvolatile fractions of MPR, colophonium and propolis need to be investigated, as they may also harbour allergenic components.

4.3 | Limitations

Our analyses were not repeated for verification. Not all peaks in the chromatograms could be identified and for some, there was some uncertainty in their identification. The percentages of the peak areas may not reflect their quantitative presence in the source material. The analytical method used by us can identify chemicals in the volatile fraction of the propolis samples only.

Author Contributions

Anton de Groot: conceptualization, visualization, writing – original draft, writing – review and editing. **Norbertus A. Ipenburg:** visualization, project administration, writing – review and editing. **Emma M. van Oers:** writing – review and editing. **Thomas Rustemeyer:** supervision, writing – review and editing. **Evelyn Calta:** conceptualization, investigation, resources.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that supports the findings of this study are available in the Supporting Information of this article.

References

1. A. C. De Groot, "Propolis: A Review of Properties, Applications, Chemical Composition, Contact Allergy, and Other Adverse Effects," *Dermatitis* 24, no. 6 (2013): 263–282, https://doi.org/10.1097/DER.0000000000000011.

2. A. C. De Groot, "*Myroxylon pereirae* Resin (Balsam of Peru)—A Critical Review of the Literature and Assessment of the Significance of Positive Patch Test Reactions and the Usefulness of Restrictive Diets," *Contact Dermatitis* 80, no. 6 (2019): 335–353, https://doi.org/10.1111/cod.13263.

3. A. C. De Groot, "Monographs in Contact Allergy, Volume II. Fragrances and Essential Oils.," *Chapter 3.126 Myroxylon pereirae resin (Balsam of Peru), pp. 527–552; Chapter 3.70 Fragrance Mix I, pp. 296–319* (CRC Press Taylor and Francis Group, 2019).

4. A. C. De Groot, "Monographs in Contact Allergy. Volume I. Non-Fragrance Allergens in Cosmetics (Part I and Part 2).," *Chapter 2.126 Colophonium, pp. 336–355. Chapter 2.385 Propolis, pp. 1042–1055* (CRC Press Taylor and Francis Group, 2018).

5. A.-T. Karlberg and L. Hagvall, "Chapter 41: Colophony: Rosin in Unmodified and Modified Form," in *Kanerva's Occupational Dermatology*, 3rd ed., ed. S. M. John, J. D. Johansen, T. Rustemeyer, P. Elsner, and H. I. Maibach (Springer Nature Switzerland AG, 2020), 608–624.

6. E. Calta, A. de Groot, E. M. van Oers, N. E. Ipenburg, and T. Rustemeyer, "Composition of Brazilian and Chinese Propolis for Patch Testing.," *Contact Dermatitis* 93, no. 1 (2025): 56–60, https://doi.org/10.1111/cod.14796. Online ahead of print.

7. G. Kocabas, N. A. Ipenburg, A. de Groot, and T. Rustemeyer, "Results of Patch Testing Propolis in the European Baseline Series: A 4-Year Retrospective Study," *Contact Dermatitis* 91, no. 5 (2024): 375–378, https://doi.org/10.1111/cod.14678.

8. E. M. Van Oers, N. A. Ipenburg, A. de Groot, E. Calta, and T. Rustemeyer, "Results of Concurrent Patch Testing of Brazilian and Chinese Propolis," *Contact Dermatitis* 92, no. 5 (2025): 344–348, https://doi.org/10.1111/cod.14748.

9. K. Piontek, S. Radonjic-Hoesli, J. Grabbe, et al., "Comparison of Patch Testing Brazilian (Green) Propolis and Chinese (Poplar-Type) Propolis: Clinical Epidemiological Study Using Data From the Information Network of Departments of Dermatology (IVDK)," *Contact Dermatitis* 92, no. 3 (2025): 209–216, https://doi.org/10.1111/cod.14701.

10. A. Antelmi, I. Trave, R. Gallo, et al., "Prevalence of Contact Allergy to Propolis—Testing With Different Propolis Patch Test Materials," *Contact Dermatitis* 92 (2025): 349–357, https://doi.org/10.1111/cod.14773.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.